Intramural papers of the month

By Greg Buchold, Deacqunita Diggs, Gabriel Knudsen, Vijay More, and Shannon Whirledge

NTP finds arsenic induces multiple cancer characteristics in lung cell model

Researchers in the National Toxicology Program (NTP) used inorganic arsenic to convert human peripheral lung epithelial (HPL-1D) cells into cells with characteristics of cancer. The work suggests that low-level arsenic exposure may directly induce changes in lung epithelial cells that could lead to adenocarcinomas. The model may be useful in studying the mechanisms of arsenic-induced lung cancer.

After 38 weeks of arsenic exposure, HPL-1D cells developed some characteristics of cancer cells, including secreting enzymes common in cancer cells, growing in serum-free medium and forming colonies, and reducing the protein expression of tumor suppressor genes. Once the cells reached this stage, the researchers renamed them chronic arsenic-treated lung epithelia (CATLE) cells.

Epithelial-to-mesenchymal transition (EMT) occurs in cells as they change into cancer cells. CATLE cells exhibited a decrease in the EMT marker protein, e-cadherin, while the metallothioneins MT1A and MT2A, and the oxidative stress response genes HMOX1 and HIF1A, increased in response to arsenic exposure.

This research is consistent with work from other groups that suggests lung adenocarcinomas likely arise from the epithelia of peripheral lung tissue. Although additional cell models are needed, this model may be used to explore arsenic-induced lung cancer. **(DD)**

Citation: Person RJ, Olive Ngalame NN, Makia NL, Bell MW, Waalkes MP, Tokar EJ. 2015. Chronic inorganic arsenic exposure in vitro induces a cancer cell phenotype in human peripheral lung epithelial cells. Toxicol Appl Pharmacol 286(1):36-43.

Mammalian milk secretion uses novel mechanism

According to scientists from NIEHS and several other academic institutions, Orai1, a calcium channel subunit, is responsible for delivering 50 percent of the calcium ions present in mammalian milk, and for signaling milk secretion through mammary cell contractility. The finding that Orai1 is required for milk ejection redefines how the process is thought to occur. The report describes this important mechanism behind the process of lactation.

Using genetically modified mice, the research team found that mice lacking Orai1 produced milk containing low concentrations of calcium. They also captured the pulsating contractions of the mammary alveoli and quantified the dependency of alveoli cells on Orai1, using state-of-the-art 3-D imaging technology. Alveoli from mice without Orai1 exhibited infrequent contractions, were less responsive to oxytocin, and were less coordinated.

These data demonstrate that calcium ions play a role in cellular signaling greater than being simply a nutritional component. The novel role of Orai1 channels in alveolar contractility also suggests the possibility of their involvement in the function and dysfunction of other exocrine tissues, including sweat glands. **(VM)**

Citation: Davis FM, Janoshazi A, Janardhan KS, Steinckwich N, D'Agostin DM, Petranka JG, Desai PN, Roberts-Thomson SJ, Bird GS, Tucker DK, Fenton SE, Feske S, Monteith GR, Putney JW Jr. 2015. Essential role of Orai1 store-operated calcium channels in lactation. Proc Natl Acad Sci U S A 112(18):5827-5832.

The origins of the ovarian theca cells

NIEHS researchers and collaborators have discovered the origins of the steroid-producing theca cells in the ovary, as well as the signaling pathways involved in cell fate specification and differentiation of the theca cell. During development, the ovary establishes follicles, consisting of the oocyte surrounded by granulosa and theca cells. This basic unit represents the foundation of mammalian reproduction, and dysfunction in any one of these cell types can result in infertility.

Using an inducible lineage-tracing model, the authors show that adult theca cells stem from two distinct progenitor populations — one from the fetal ovary and the other from early embryonic tissue, called mesonephros. These two populations are present in the adult ovary at different numbers and maintain a lineage-specific transcriptome. Ovary-derived theca cells exhibit higher expression of genes implicated in cell growth and proliferation, while those derived from the mesonephros are enriched for genes that regulate steroidogenesis.

Regardless of their origin or adult function, the same signaling pathway is responsible for theca cell differentiation from the two populations. Components of the Hedgehog signaling pathway, Indian hedgehog (Ihh) and Desert hedgehog (Dhh), which are critical to normal embryonic development, induce expression of Gli1, the marker of progenitor theca cells. The production of Hedgehog signals from the granulosa cells is achieved through oocyte-derived factor, indicating that theca cell development in the ovary is a result of the coordinated signaling between many cell types. Understanding the complex, multicellular process in which theca cells arise may shed light on disorders resulting from aberrant theca cell development, such as polycystic ovary syndrome and premature ovarian failure. **(SW)**

Citation: Liu C, Peng J, Matzuk MM, Yao HH. 2015. Lineage specification of ovarian theca cells requires multicellular interactions via oocyte and granulosa cells. Nat Commun6:6934. (Story)

New biomarkers could screen endocrine-disrupting chemicals

A research team led by NIEHS scientists has identified a group of more than 100 genes capable of identifying endocrine-disrupting chemicals (EDCs), which are compounds that interfere with the hormone system of an organism. Using these genes as biomarkers could not only help uncover the mechanisms that EDCs use to impact public health, but could also serve as a secondary screen for the Tox21 high throughput project at NTP.

EDCs can behave like naturally occurring estrogens, negatively altering the balance of signaling pathways that are normally triggered by estrogen. The researchers tested the estrogenic effects of substances on mouse uterine tissue by measuring the increase of DNA synthesis in epithelial cells. At the end of 24 or 72 hours of treatment, they evaluated the weight of uterine tissue and used the data to indicate whether the estrogenic substance was long-acting, which resulted in a weight increase, or short-acting, which brought little or no weight increase.

Using microarrays and computational tools the scientists analyzed how long-acting or short-acting estrogens impact thousands of genes in the uterus. They selected more than 100 genes to use as a biomarker panel to distinguish chemicals that mimicked long-acting (estradiol or diethylstilbestrol) versus short-acting (estriol) estrogens. The novel chemical diarylheptanoid D3, derived from a plant in the ginger family used by postmenopausal women in Thailand, was identified through the assay as a short-acting xenoestrogen similar to bisphenol A and HPTE. **(GB)**

Citation: Hewitt SC, Winuthayanon W, Pockette B, Kerns RT, Foley JF, Flagler N, Ney E, Suksamrarn A, Piyachaturawat P, Bushel PR, Korach KS. 2015. Development of phenotypic and transcriptional biomarkers to evaluate relative activity of potentially estrogenic chemicals in ovariectomized mice. Environ Health Perspect 123(4):344-352.

Long repetitive sequences drive some types of mutations

NIEHS researchers have demonstrated that insertion-deletion, or indel, mutation rates increase 100,000-fold with increasing homonucleotide run length, using yeast strains with combinations of defects in their mismatch repair (MMR) machinery and DNA polymerases. Such runs play important roles in chromatin organization, gene expression, and chromosome replication. Mutation rates are used to calibrate molecular clocks, assess human tumors, and identify genetic variants in human diseases. Prior to this research and the work of other NIEHS scientists, many researchers assumed the rates were similar across the genome.

These small indels, driven by DNA strand slippage during replication, are usually repaired by both MMR and exonucleolytic proofreading (PR). MMR defects allowed indels, especially in long DNA repeat tracts, which are the source of the microsatellite instability that is a hallmark of MMR-defective tumors. In contrast, the researchers showed that PR defects selectively increase indel rates in short repeat tracts. These data are especially useful given that coding sequences contain higher proportions of short tracts than noncoding regions of DNA, leading to the accumulation of more coding mutations, with likely ultimate outcomes of disease or carcinogenesis. Should the indel pattern hold in future studies of tumors containing PR defects, indels in short runs could be diagnostic in the way that microsatellite instability is for mismatch repair-defective tumors. **(GK)**

Citation: Lujan SA, Clark AB, Kunkel TA. 2015. Differences in genome-wide repeat sequence instability conferred by proofreading and mismatch repair defects. Nucleic Acids Res 43(8):4067-4074.

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